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U.S. Army

HEADQUARTERS RLH/dr
406TH MEDICAL GENERAL LABORATORY, Tokyo
APO 500, c/o Postmaster
San Francisco, California

16 January 1950

SUBJECT: Technical Report for December 1949

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TO: The Surgeon General, Department of the Army, Washington 25, D.C.

1. In compliance with Paragraph 4.e., Circular 1C6, GHQ, FEC, 14 November 1947, the technical report of this unit for December is submitted.

2. Administration: a. Personnel - (1) The following changes in officer and DAC personnel occurred:

Name	Qualification	Change	Asgd. Section
Capt. Charles J. Wilson, MSC	Supply Officer	Arr. 16 Dec 49	Supply
Capt. Lewis A. Palmer, MC	Pathologist	Dep. 3 Dec 49	Pathology
Dr. Perry Elrod, GS-12	Bacteriologist	Arr. 3 Dec 49	Bacteriology
Miss Margaret Recksiek, GS-6	Med. Technician	Arr. 30 Dec 49	Bacteriology

(2) Enlisted Personnel - This organization had 3 enlisted gains during the month and one enlisted loss by transfer to ZI for separation. Three enlisted men were attached pending assignment 2 January 1949. Six enlisted students reported on 30 December for 10 days training from Laboratory Technicians School. At the end of the month enlisted strength was 68 assigned and 9 attached.

(3) Foreign National Personnel - No change, strength remained at 8.

(4) Japanese National Personnel - There were 2 gains during the month and 3 losses. End of the month strength was 89.

b. Field Trips - The following field trips were made during the month:

Place	Section	Officer	Date
Korea	Virus	Capt. Whatley and Major Burns	23 November - 8 December
PHILCOM	Virus	Major Votaw	3 December - 14 December

c. Routine Work Performed - In the Medical Illustration Section there were 16 photographic prints made; 3 plates were made; 7 signs were painted; 6 maps were made and 2932 photostats were made.

d. Difficulties Encountered - A Medical Equipment Repairman was assigned to Supply on 24 December. Space in which he can work has not been discovered as yet.

e. Miscellaneous - Two T/O and E medical laboratory trucks with equipment arrived on 30 December.

3. Routine: As in the past, tabular presentation of routine work is not included. Certain points of general interest are mentioned under section headings.

4. Medical Zoology: a. Laboratory Infections in the Snail - Shedding tendencies of cercariae of S. japonicum in experimentally infected snails are being studied. During the summer of 1949, large numbers of O. nosophora snails were experimentally infected with the miracidium of S. japonicum. Laboratory raised snails as well as those from the field were infected. Under laboratory conditions the development of these infections has been very slow, but within the past month some of these snails have been found to contain mature cercariae. To date a series of 9 experimentally infected snails have been isolated and set up daily in glass sputum cups for shedding. Natural infections are used as controls for recognizing the suitability of water and temperature.

b. Preparation of Antigens - Two cats which had been exposed to cercariae of S. japonicum during the month died. One yielded 428 and the other 186 adult worms. These were frozen and thawed, lyophilized and stored in sealed ampules on dry ice. Two hundred adult Clonorchis sinensis present in the second cat were similarly treated.

c. Publications - Notice of publication of the following articles has been received:

(1) The Control of Schistosomiasis Japonica. V. Studies on the Penetration of Various Types of Unimpregnated Uniform Cloth by Cercariae of Schistosoma japonicum, by Hunter, G.W. III, Bennett, H.J., Fry, N.H., Sec, J., and Greene, E. Am. J. Trop. Med. 29(5):723-737, 1949.

(2) Parasitological Studies in the Far East. VII. An Epidemiological Survey in Southern Korea, by Hunter, G.W. III, Ritchie, L.S., Chang, I.C., Rolph, W.D. Jr., Mason, H.C., and Szweczak, J.T. J. Parasit. (Suppl.) (Abstract), 1949.

(3) Experiments with Bird and Human Schistosomes, by Hunter, G.W. III, Ritchie, L.S., Tigertt, W.D., Lin, S., Pan, C., and Tanabe, H. J. Parasit. (Suppl.) (Abstract), 1949.

(4) Protection Experiments with Copper Oleate Ointment against Schistosomiasis, by Kaufman, E., Hunter, G.W. III, and Pan, C. J. Parasit. (Suppl.) (Abstract), 1949.

(5) Notes on the Course of a Pinworm Infection by McMullen, D.B. J. Parasit. 35(5)1542-543, 1949.

(6) Seasonal Studies on Schistosoma japonicum in the Intermediate Host Oncomelania nosophora, by McMullen, D.B. J. Parasit. (Suppl.) (Abstract), 1949.

(7) Parasitological Studies in the Far East. VI. An Epidemiological Survey of Kyushu Island, Japan, by Ritchie, L.S., Hunter, G.W. III, Pan, C., Yokogawa, M., Szewczak, J.T. J. Parasit. (Suppl). (Abstract), 1949.

5. Chemistry: a. Clinical Chemistry - The routine work load was similar to that reported for October with 511 examinations on [illegible] specimens submitted. This volume is despite the holiday season, and decision to change the work month for reporting purposes from 25th to 25th rather than 1st to 30th. Apparently an increased incidence in disease or interest led to an eight-fold increase in requests for cholesterol-ester determinations.

b. Toxicology - Little change in work-volume occurred from last month, material being submitted on 12 autopsies. In fifty per cent of cases ethyl alcohol was present. Case R.L. was the most interesting of the month. The carbon monoxide content of the blood was found to be 89 per cent of blood saturation. Goodman and Gilman state that a concentration of 70-80 per cent of carbon monoxide of blood saturation is sufficient to cause death. Confirmation of the presence of carbon monoxide was obtained in three qualitative tests namely, alkaline hematin, blood plus ammonium sulfide plus acetic acid, and the method of Sayers and Yant.

c. Miscellaneous Analyses - As in previous months, a variety of miscellaneous substances were submitted for identification, specification requirements, detection of narcotics, etc.

Halazone tablets obtained from 54 bottles were analyzed this month. These were the products of the Empire Chemical Co., Abbott Laboratories, The Drug Products Co., and Kentlee Co., Inc. Of the ten (10) lots analyzed only three (3) were found to be still usable, these being, Abbott Laboratories codes G504T333, G504T335, and G502T451 which contained 0.90, 0.91, and 0.98 mg. of available chlorine per tablet, respectively.

d. Barbiturate Analysis - Study along these lines continues; the following facts have now been determined:

(1) Using a Coleman Spectrophotometer maximum absorption of light by an aqueous cobalt solution occurred at 510 mu.

(2) The C-T curve follows the Beer-Lambert Law.

(3) Concentrations up to 1.2 gm. of cobalt chloride in an aqueous solution can be determined sprectrophotometrically.

(4) A standard solution can be prepared directly from the hydrated salt ($COCl_2 6H_2O$) without further gravimetric determination.

6. Pathology: a. Routine - During the month of December 260 surgical specimens were received. Malignancies encountered were as follows:

Basal cell carcinoma of skin	2
Secondary carcinoma in cervical lymph nodo, poorly differentiated	1
Secondary carcinoma from chest wall	1

During the month 48 autopsies were recorded, of which number 18 were performed by members of the staff of this section at the Tokyo General Hospital.

b. Liaison with Other Agencies - During the month 3 surgical pathology conferences were held with the Surgical Service, Tokyo General Hospital. Two clinical pathologic conferences were given by Dr. [illegible] Humphrey, Consultant in Pathology to the Surgeon General, one at the 361st Station Hospital and one at the 155th Station Hospital. A lecture on fat embolism and lipoid disease of the lungs was given by Dr. Humphrey at Tokyo General Hospital. Our section was visited by Dr. Humphrey for several days and opportunity was taken to consult with him on several cases and to orient him on the tissue pathology situation in the Far East Command.

During the month 124 surgical specimens and 20 autopsies were transmitted to the Armed Forces Institute of Pathology. A supply of metal containers has been arranged for to allow more satisfactory means of packaging.

7. Serology: a. Rh factor - In order to locate possible future sources of blood of known Rh composition the blood group of 67 American and 25 Japanese personnel of the 406th Medical General Laboratory were determined. Twenty-eight selected blood groups were analyzed for the various Rh subtypes. With the exception of CdE (Rh'Rh''), at least one individual in each Rh subtype was identified. Lack of Hr antiserum prevented analysis of Rh negative bloods for Hr subtypes.

b. Rh_0 Antibodies - 117 sera were examined. Antibodies were detected in 4 undiluted sera, 3 gave titers, 105 demonstrated no antibodies and 5 were unsatisfactory for testing.

c. Cardiolipin - Doubtful microflocculation reactions again occurred with excessive frequency in December. As noted previously the flocculation antigen reacted slightly with some sera which were unreactive to Wassermann antigen.

d. Serologic Surveys - Continuing surveys in conjunction with Eighth Army Surgeon's venereal disease program constituted 1175 examinations or 14.5% of the total cardiolipin microflocculation examinations.

8. Bacteriology: a. Diphtheria - From 21 November to 20 December this laboratory isolated Corynebacterium diphtheria from three patients, one soldier and two dependent civilians. Culture surveys were made as a part of the epidemiologic study of these cases.

(1) The first case was a child who died from the infection after delayed administration of antitoxin. This section isolated virulent Corynebacterium diphtheriae from cultures begun during life. Cultures taken at autopsy were negative for C. diphtheriae. The organism recovered from cultures was a virulent gravis type. This patient had originally been hospitalized at the 5th Station Hospital, Johnson AFB. A contact survey of 50 contacts, both Occupation and Japanese, was fruitless in that no C. diphtheriae

was recovered.

(2) The second case was another dependent child, hospitalized at Tokyo General Hospital from the Grant Heights Dispensary. The child was not ill from diphtheria, but was admitted with a sore throat and no severe symptoms. Throat cultures were negative for C. diphtheriae. However, a nasal culture from the child produced a virulent gravis-type C. diphtheriae. A survey of contacts was made with 47 throat cultures being taken from Occupation and Japanese persons. From two Japanese employees, one a maid recently discharged from the patient's home, virulent and weakly-virulent mitis type C. diphtheriae was recovered. The virulent strain was from the former employee. No other persons in the survey yielded positive cultures.

(3) The third case of diphtheria was a soldier who was diagnosed at the Clr. Co., 15th Med. Bn., and who was treated at the 361st Station Hospital. From this soldier a non-virulent mitis type C. diphtheriae was isolated. The throat culture survey of 16 contacts in the soldier's case revealed no carriers or cases infected with C. diphtheriae.

b. Enteric Pathogens - (1) The Isolation of Three Serotypes of S. paradysenteriae from a Single Case - On 8 December plates of S.S. and EMB agar containing non-lactose fermenting colonies were received from the 155th Station Hospital. Four colonies were picked from the S.S. agar, two from the EMB to Kligler (1% sucrose added) slopes. Five proved to have the presumptive biochemical characteristics of the genus Shigella; all agglutinated in Shigella group A sera. The use of specific typing sera proved one to be type 103, two were VZ and two Boyd 88.

All organisms were purified by restreaking and the biochemical and serological properties checked. The types were verified and biochemical tests found consistent. Of some interest was the fact that those organisms typed as Boyd 88 were gas producers in both dextrose and mannitol; thus Manchester strains.

A repeat stool specimen nine days later found all selected colonies to be type VZ. Isolation, however, of the Manchester strain was also accomplished by the 155th Dispensary.

To our knowledge there have been no cases of bacillary dysentery in which three serotypes of Shigella paradysenteriae have been isolated.

(2) Variation of a Shigella Serotype in Vivo - Variation within the many serotypes of the genus Shigella has been observed and described on numerous occasions (Takita, Boyd and Veasie). The exact nature of these changes is still a matter for argument although Boyd's idea of a loss variation seems to explain best the majority of such phenomena. In so far as we can determine, such variations have resulted from old stock cultures or from parent cultures which threw off variants, usually in two directions. We have recently, however, observed this loss variation in a long-standing case of human dysentery.

On 12 September 1949 a stool culture was submitted from Benjamin Coxwell by the 128th Station Hospital. Over a period of about six weeks, during which time the man was hospitalized, twelve stool specimens were submitted. From the first five of these a pathogen was isolated and identified as S. paradysenteriae type W. From the following six specimens an organism was obtained which

reacted in a manner different from the originally isolated organisms. From the twelfth stool no enteric pathogen was obtained.

Comparative agglutination studies proved that the first organism isolated (W-A) was specific in its reactions, there being little evidence of cross-agglutination relationships with other Flexner strains. On the other hand the second type, W-B, showed a good mosaic of group antigens, reacting with the majority of the Flexner types in an avid fashion. It was further demonstrated by reciprocal absorption experiments conducted with antisera prepared against the two types that W-A actually was richer in specific substance, while W-B had lost much of this antigen and had either gained or had unmasked a number of group antigens. Preliminary experiments indicate that the unmasking hypothesis probably prevails. This has an exact analogy in Boyd's work with the 103A-103-B variation.

c. Antistreptolysin Studies - (1) Antistreptolysin level of normal human serum - For comparison with antistreptolysin titer in clinical cases, it was considered desirable to have the antistreptolysin level of normal human serum. Blood samples of ten apparently healthy individuals working in the Bacteriology Section were secured during this month and were run for antistreptolysin titer against streptolysin "O", streptolysin S and streptolysin S'. Results are summarized in the following table:

Serum No.	Antistreptolysin "O"	Antistreptolysin S	Antistreptolysin S'
1	80 u./ml.	2 M.H.D./ml.*	16 M.H.D./ml.
2	80 "	4 "	16 "
3	40 "	4 "	16 "
4	160 "	4 "	16 "
5	160 "	4 "	8 "
6	40 "	2 "	16 "
7	160 "	2 "	16 "
8	40 "	4 "	8 "
9	80 "	No titer	8 "
10	40 "	No titer	8 "

* Since standard antisera for streptolysin S and streptolysin S' are not available, the titer of antistreptolysin S and S' is expressed in antihemolytic activity against minimal hemolytic dose.

As may be seen from the table, the antistreptolysin level of normal human serum is about 80 units per ml. Since common respiratory injections show season variation in incidence it will be necessary to determine if there is a similar seasonal variation of the level of antistreptolysin in normal subjects. This investigation will be continued through the seasons.

(2) Possible Identification of streptolysin S and streptolysin S' - In the report of "Properties of certain rapidly acting bacterial toxins as illustrated by streptolysin O and S" (Bact. Rev., 12:195-202, 1948), Bernheimer states that "In all respects studied, however, the nucleic acid hemolysis is identical with streptolysin S." In this laboratory we have examined: A) The pH range for optimal hemolytic activity and B) the degree of stability

in varying temperature, and found that S and S' have exhibited apparent difference in the latter point. Experiments were set up as follows:

Media for S-lysin

Bacto-beef (veal	50 g.
Protcose peptone (Difco)	20 g.
NaCl	2 g.
Na_2HPO_4	1 g.
Distilled Water	1000 ml.

pH to 7.6 and autoclave 15 lbs for 15 minutes

Supplement: horse serum ..... 20% (final aseptically added

Incubation 37°C 6 hours

Media for S'-lysin

Bacto-beef (veal)	50 g.
Protcose peptone (Difco)	20 g.
NaCl	2 g.
Na_2HPO_4	1 g.
Distilled Water	1000 ml.

pH to 7.6 and autoclave 15 lbs for 15 minutes

Supplement: nucleic acid ..... 1% (Final) aseptically added.

Incubation 37°C 20 hours

Strains: Streptococcus pyogenes (Blackmore and Lang)

Differences in the two media are only in supplement and incubation time. Blackmore and Lang strains are known to produce only streptolysin S and there was no streptolysin O detected in the culture of the above media.

A) The pH range for optimal hemolytic activity of streptolysin S and S'. Phosphate buffer was used for pH range between 6.4 to 7.8 and borate buffer was used for pH above 8.0. The relationship between hemolytic activity of streptolysin S and pH range is illustrated in Fig. 1 (Incl. 1), which indicates optimal pH for streptolysin S is around pH 8.2. The relationship between hemolytic activity of streptolysin S' and pH range is illustrated in Fig. 11 (Incl. 2), which indicates optimal pH for streptolysin S' is also around pH 8.2. The relationship between hemolytic activity and pH range shows similar pattern for both streptolysin S and S'.

B) The stability of streptolysin S and S' at varying temperatures - Three steps of temperature were taken for convenience, that is, 37°C in water

bath, 23°C in room temperature and 4°C in refrigerator. Stability of streptolysin S and S' at 23°C and 37°C is illustrated in Fig. III, (Incl. 3), which shows a marked difference between streptolysin S and streptolysin S'. Streptolysin S' does not show a decrease in activity for 21 hours at 37°C. and for 112 hours at 23°C, whereas streptolysin S shows a rapid decrease in activity for 4 hours at 37°C and for 40 hours at 23°C. Although streptolysin S is more stable at 40°C for 10 days than at the above temperature, it is not so stable as streptolysin S' at 4°C, as illustrated in Fig. IV (Incl 4).

9. Entomology (Overwintering Survey and Collections): a. Monthly activities of the Entomology Subsection have centered mainly on an overwintering survey of mosquitoes during the month of December. One hundred forty-five stations representing various habitats were visited during the month, a summation of which appears in Table I. (Incl. 5). During this period a total of 3,209 living adult mosquitoes were taken, the great majority from caves and bomb shelters, small numbers being taken from horse stables, dairy barns, chicken houses, sheds and barges. The yields from these collections are indicated in Table 2 (Incl. 6). It is of interest to note that 99% of all collections of overwintering adult mosquitoes were Culex pipiens. From these adult collections 1,340 Culex pipiens females and 5 Culex hayashii females have been tubed for virus isolation attempts; 96% female and 120 male Culex pipiens were used for hibernation studies with JBE.

b. In addition to adult collections, 7 collections of mosquito larvae and/or pupae were made during the month. Five collections were made from tombstones in cemeteries yielding 5 collections of Aedes togoi and 2 of Aedes japonicus. No adults of either species were taken during this month. A sixth larval collection yielded Aedes togoi from a cistern, and the seventh larval collection contained Culex pipiens from a bomb shelter where large numbers of adults of the same species were taken also.

10. Virus and Rickettsial Section: a. JBE in Korea - Additional material and data have been obtained for more satisfactory explanation of the recent epidemic. At the time of earlier investigation there was no regular air travel between Japan and Korea. Accordingly serum had to be kept in the freezing compartment of electric refrigerators, and returned to this laboratory in wet ice. Power failure and shut-downs frequently prevented maintaining freezing temperatures in Seoul. Herein may lie an explanation for the small number of cases with appreciable CF titers in "convalescent" cases. Establishment of a regular weekly air schedule between Japan and Korea has now allowed regular shipments of dry ice to field investigators for more satisfactory preservation of C.F. antibodies.

Blood specimens were collected from a group of children in an orphanage, and from Korean soldiers whose history indicated moderate variation in residence in Korea. Specimens were collected immediately before and nine days after a stimulating dose of JBE vaccine. In addition animal blood was obtained as follows:

a. 21 sera from two flocks of chickens in Seoul.
b. 24 sera from horses of the Korean Cavalry Regiment.
c. 14 sera from war dogs.

d. 17 sera from swine at a local slaughter house.
e. 31 sera from cattle on a farm 7 miles from Seoul.
f. 20 sera from sheep at the National Vaccine Laboratory.

A total of 341 human and animal sera were obtained. Complement fixation and neutralization tests are in progress.

b. Serologic survey in Hokkaido - In September and October 1949 an opportunity presented for a preliminary serum survey of Hokkaido. This northernmost island of Japan has long been recognized as relatively free of the annual onslaught of JBE in horses and humans. The epidemic of 1948, however, was reflected by an annual rate of 0.2 per 100,000. Information was desired as to whether there had been an inapparent dissemination of the virus during that year. A total of 192 sera were collected from four areas: (1) Wakkanai, (2) Mombetsu, (3) Asahigawa, and (4) Obihiro. Blood was collected, serum separated and frozen, and specimens subjected to complement-fixation and neutralization tests.

Complement fixation showed one four-plus reaction in a 1:4 dilution, one anticomplementary serum, and one non-specific reaction to antigen and normal mouse brain in 1:4 dilution. The remaining 189 were negative. Results of neutralization tests are shown by age group in Inclosure 7. When compared with results obtained in 1946 in a similar survey in Sapporo (southern Hokkaido), no increased dissemination of virus in 1948 appears to have occurred. Further breakdown by geographic location suggests no limitation of the disease to any particular portion of the island.

c. Influenza - In early December, roughly 4000 cases of a respiratory infection were reported in Kagawa prefecture in a group of approximately 100,000 Japanese.

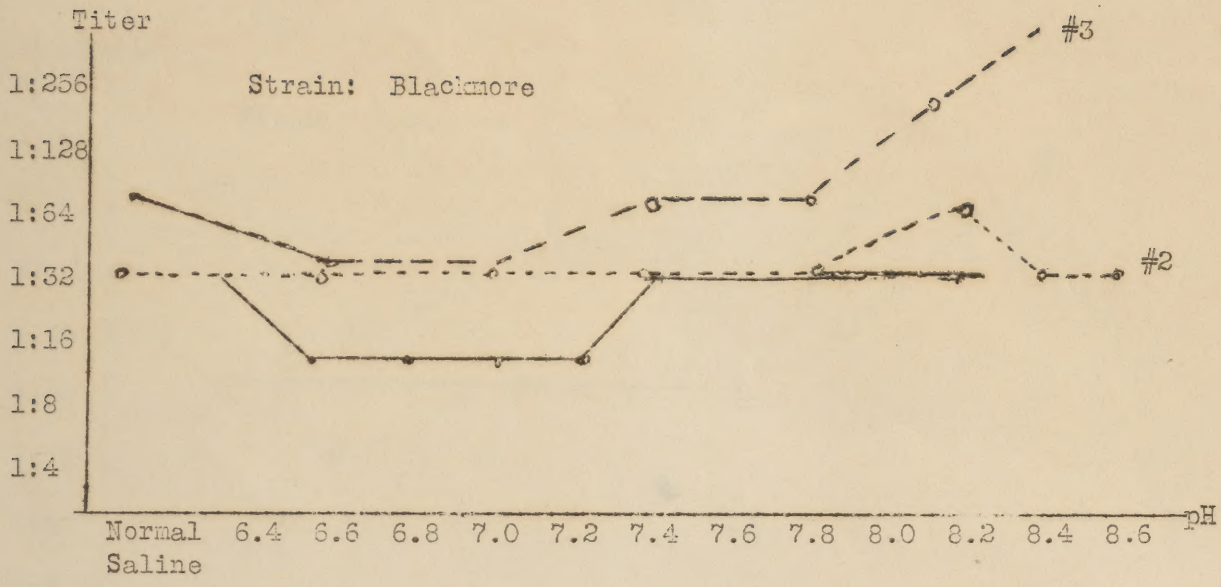
Paired serum specimens from twenty Japanese Nationals were tested for rise in influenza virus agglutination-inhibition antibodies. Only 3 of the patients gave positive results. Two had 8-fold rises in agglutination-inhibition antibodies and one had a 6-fold rise in antibodies to Lee strain of influenza.

Of 8 paired serum specimens from American suspects tested for rise in influenza virus agglutination-inhibition antibodies 7 gave negative results. One patient in this group gave a 4-fold rise to FM-1 strain of influenza.

d. Undetermined Virus Infection in the Philippines - In October and November an unusual infection of some twenty military personnel and dependents were reported. These cases manifested typically a short febrile illness (about 3 days) with nuchal rigidity, headache and pleocytosis. Attempts at virus isolation by the local laboratory were unsuccessful. Information and convalescent sera were submitted to this laboratory. Additional local investigation has been accomplished with more serum and information obtained. This material and data will be forwarded to AMDR&GS for virus study, particularly devoted to possibility of encephalomyocarditis and/or virus of the Coxsackie group being responsible.

R. L. HULLINGHORST
Lt. Colonel MC
Commanding

7 Incl
Fig. I
Fig. II
Fig. III
Fig. IV
Mosquito Survey - Table I
Mosquito Data - Table II



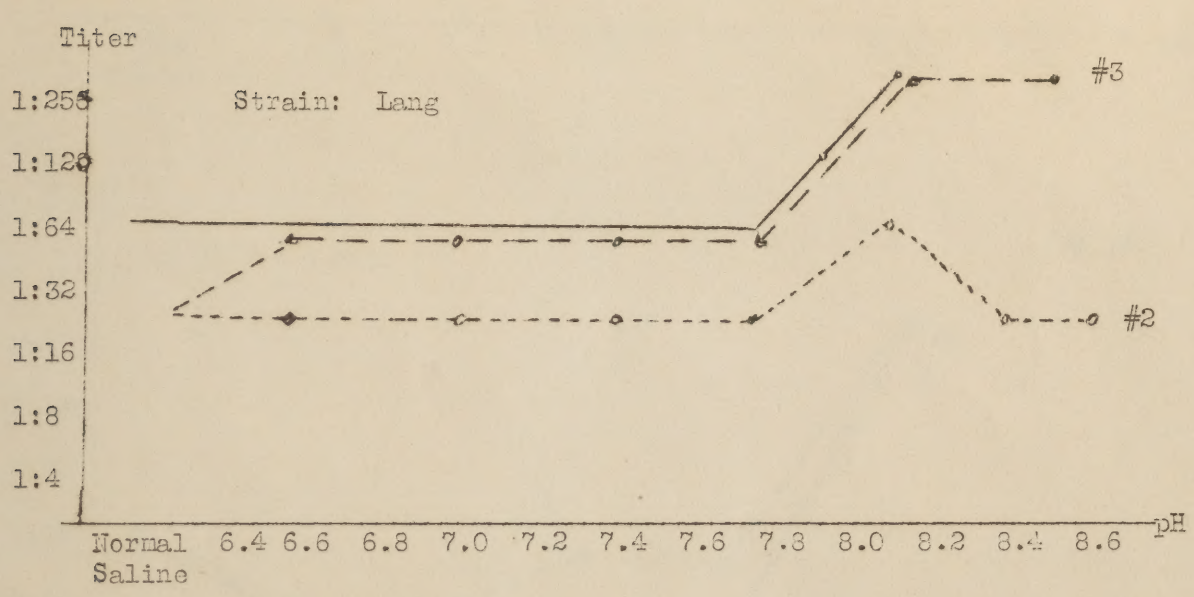
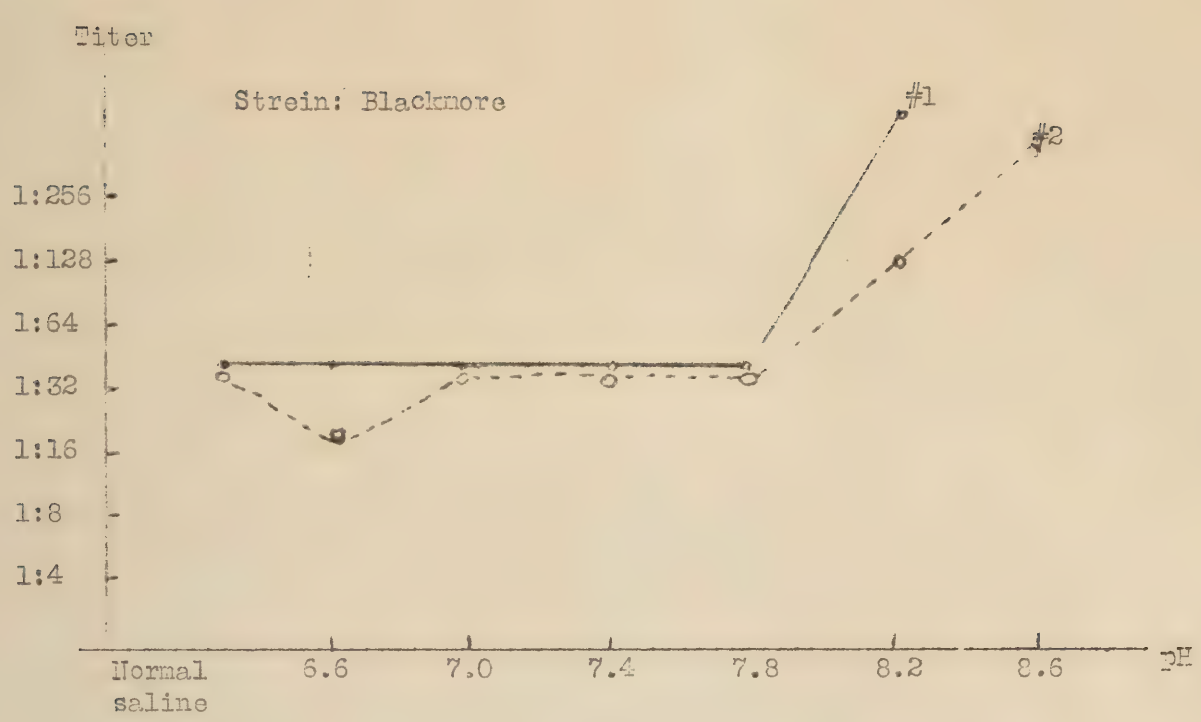


Fig. I. pH Range for Optimal Hemolytic

Activity of Streptolysin-S

Incl #1



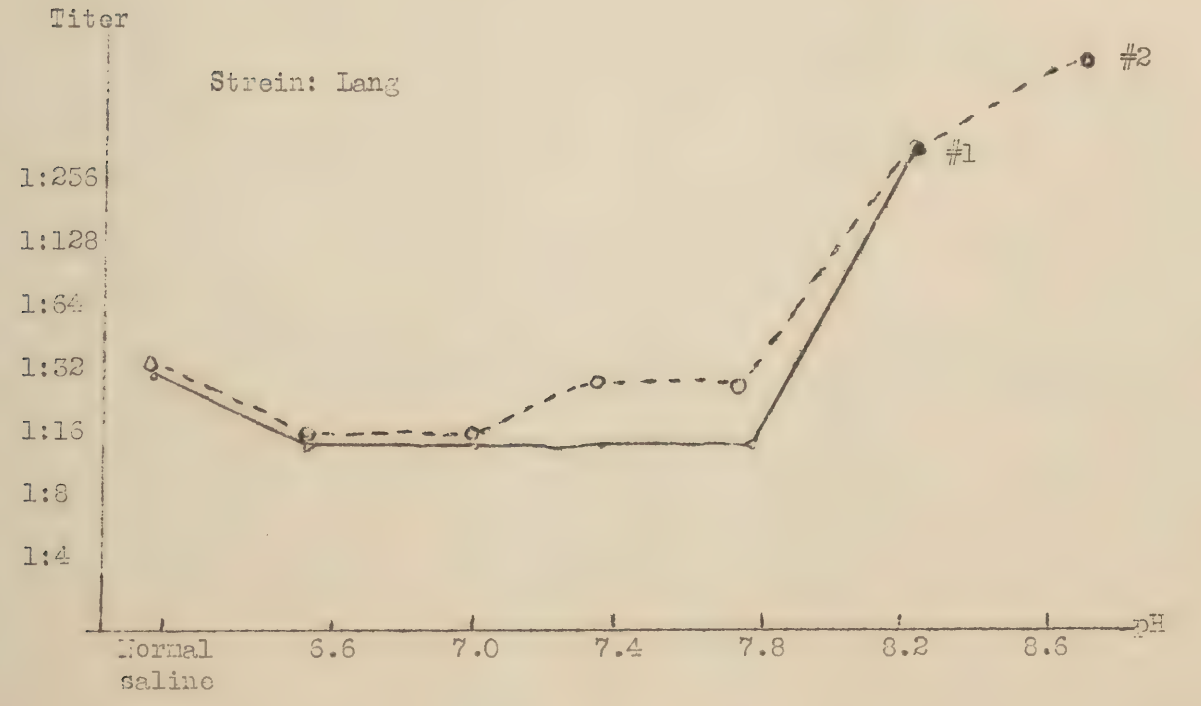
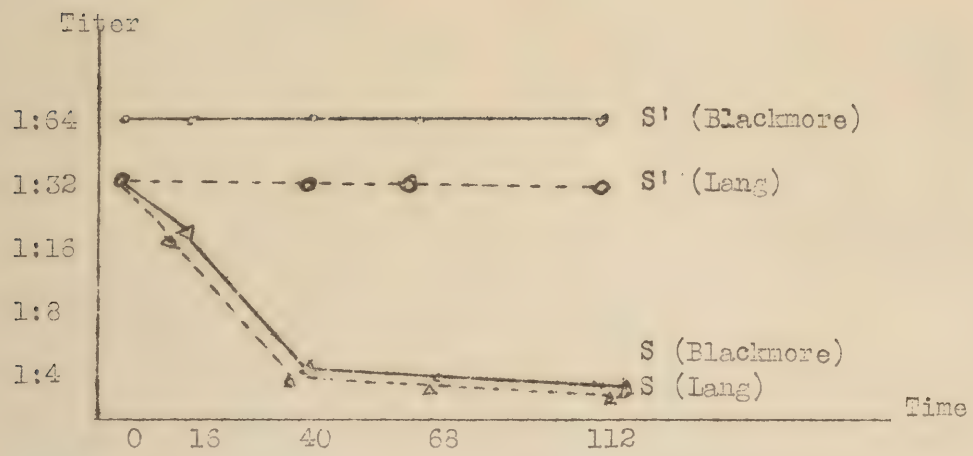


Fig. II pH Range for Optical Homolytic

Activity of Streptolysin-S'

Incl. #2

At 23°C (Room Temperature)



At 37°C (Water bath)

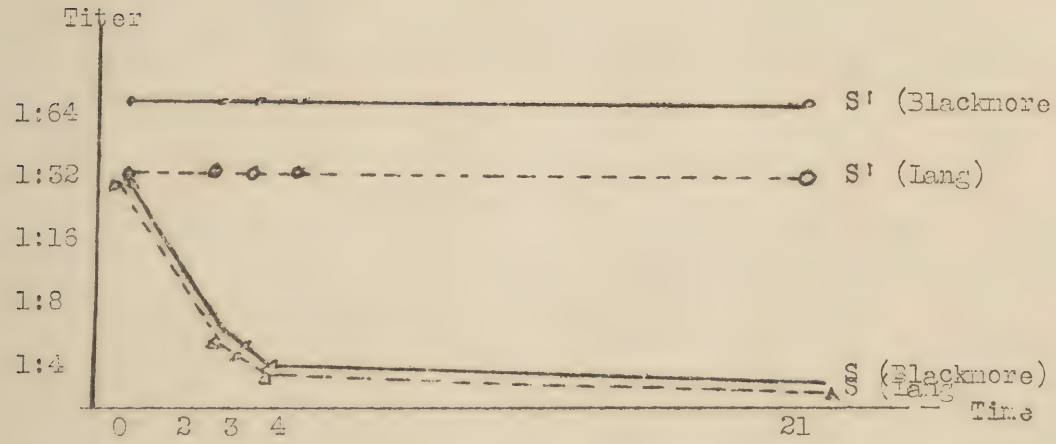
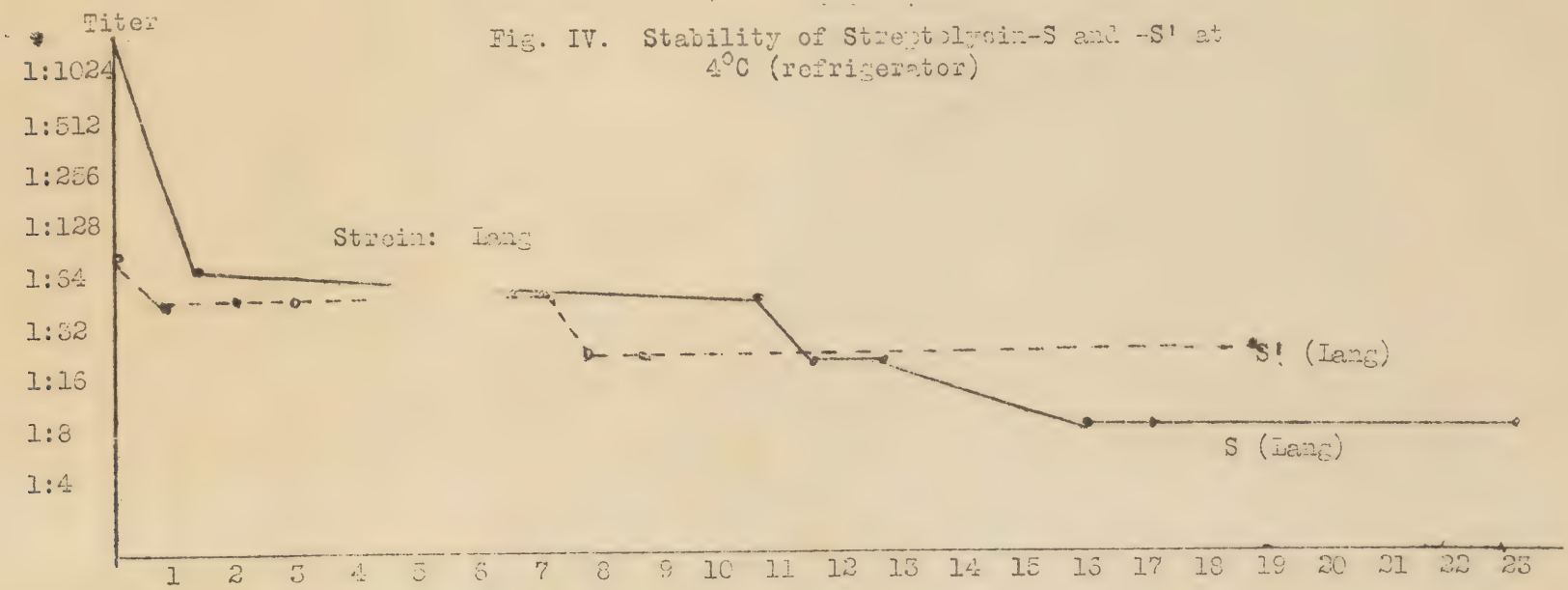


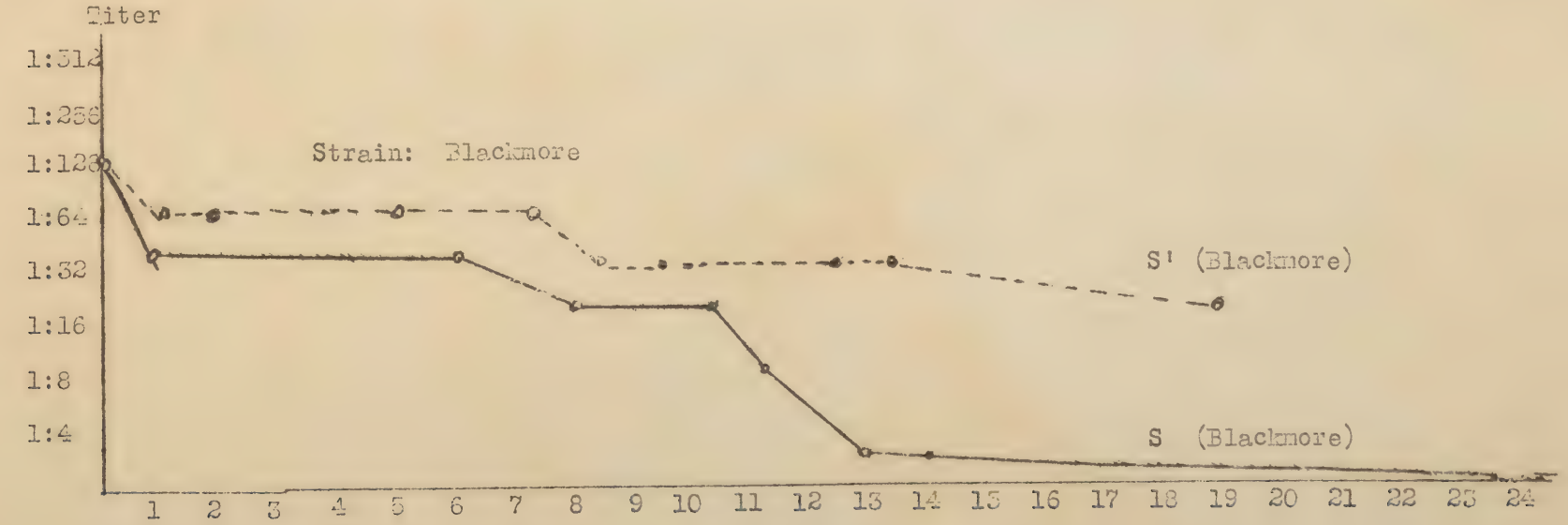
Fig. III Stability of Streptolysin-S and S'

at 23°C and 37°C

Incl #3

Fig. IV. Stability of Streptolysin-S and -S' at 4°C (refrigerator)





Incl #4

TABLE I

SURVEY OF RESTING STATIONS
for
OVERWINTERING ADULT MOSQUITOES
December 1949

Type Habitat	No. Visited	Total No. Animals or Fowl	Total No. Mosquito Collections
Horse Stables	44	168	2
Dairy Barns	8	92	1
Goat Farms	2	40	0
Pig Pens	13	204	0
Chicken Houses	5	870	1
Caves and Bomb Shelters	39	—	19
Sheds	21	—	1
Benjos	13	—	1
TOTALS	145	1374	25

Incl #5

TABLE II

COLLECTIONS OF OVERWINTERING ADULT MOSQUITOES

	No. Collections	C. pipiens M	C. pipiens F	C. hayashii M	C. hayashii F	C. mimeticus M	C. mimeticus F	C. orientalis M	C. orientalis F	TOTALS M	TOTALS F
l Bomb rs	10	411	2680	0	18	0	3	0	8	411	2709
ables	2	0	12	0	0	0	0	0	0	0	12
ns	1	0	3	0	0	0	0	0	0	0	3
louses	1	7	2	0	0	0	0	0	0	7	2
	1	20	15	0	0	0	0	0	0	20	15
	1	15	15	0	0	0	0	0	0	15	15
s	25	453	2727	0	18	0	3	0	8	453	2756
								GRAND TOTAL			3209

NEUTRALIZATION INDICES BY AGE GROUPS

Age Group	Hokkaido 1949		Sapporo 1946	
0-4	0/1	0%	1/38	3%
5-9	0/16	0%	2/40	5%
10-14	1/58	2%	0/40	0%
15-19	0/26	0%	2/42	5%
20-39	11/51	21%	2/20	10%
40-59	4/24	17%	4/19	20%
60-	0/2	0%	7/41	17%
TOTAL	16/178	5.8%	18/240	8.6%

(The numerator denotes sera with neutralization index of 50 and higher. The denominator denotes number of sera tested).

Incl #7

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